In vitro culture of *Anthurium andraeanum*

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The vegetative propagation of *Anthurium andraeanum* Lind. is beset with many problems. To simplify the propagation procedure the *in vitro* culture of this plant was re-examined. Contrary to work previously done it was found that a single medium could fulfill all the explants cultural requirements. The efficiency of the technique using differing light intensities, culture media and plant organs as explants was established. Plantlet regeneration was attained using a modified Murashige & Skoog culture medium. Plantlets were grown in a culture environment of 25 ± 2°C with a 16-h light: 8-h dark cycle at an intensity of 27 μE m^{-2} s^{-1}.

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Verskeie probleme word ondervind met die vegetatiewe voortplanting van *Anthurium andraeanum* Lind. Om hierdie voortplantingsprosedure te vereenvoudig is die weefselkultuur van die plant herondersoek. In teenstelling met vorige werk, is daar gevind dat 'n enkele medium aan al die kultuurvereistes van die eksplante kon voldoen. Die effektiwiteit van die tegniek met verskillende ligintensiteite, kultuurmedia en plantorgane as eksplante is bepaal. Plantregenerasie is verkry deur gebruik te maak van 'n gemodifiseerde Murashige & Skoog kultuurmedium. Plantjies is by 25 ± 2°C met 'n 16-uur lig: 8-uur donker-siklus en 'n ligintensiteit van 27 μE m^{-2} s^{-1} gekweek.

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Introduction

Anthurium andraeanum Lind., an outbreeding plant, is propagated by seed. As a result the progeny is very heterogeneous. Standard propagation methods such as dividing old plants or taking cuttings and suckers from the rhizome are very slow and unprofitable. Hence it became necessary to develop a rapid vegetative means of propagating anthuriums. Pierik *et al.* (1974) first reported on the *in vitro* culture of *Anthurium andraeanum*. Following these experiments where callus was used for micropropagation, a number of subsequent experiments described modifications to the original culture technique. These involved the use of liquid media for callus multiplication (Pierik 1975); modifications in applied hormone levels to achieve better growth (Pierik *et al.* 1979; Kunisaki 1980), and the elimination of intermediate callus formation (Leffring & Soede 1979).

This study was initiated in an attempt to improve on earlier procedures and to adapt them to South African conditions.

Materials and Methods

Adult plants of *Anthurium andraeanum* Lind. were used for the experiments. These plants were grown in a greenhouse under natural light conditions during summer. Explants derived from leaves, petioles, spathe, spadix and roots, were swabbed with 80% ethanol. The explants were sterilized in 1% NaOCl (containing two drops of Tween 20) for 30 min and then washed for three 30-min periods in sterile distilled water. The explants were then transferred to the respective culture media. Five media were screened during these experiments, the constituents of which are listed in Table 1. Ten cm^3 aliquots of media were dispensed in culture tubes and sealed with Cap-O-Test caps. Twenty replicates were used for each treatment. Explants were grown in a culture environment of either 25 ± 2°C or 20 ± 2°C; with continuous darkness, a 16-h light: 8-h dark cycle, or continuous light with an intensity of 27 μE m^{-2} s^{-1}.

Once the optimal medium had been ascertained, all subsequent experiments were performed using the modified Murashige & Skoog medium supplemented with 5,0 mg dm^{-3} BA, and 0,1 mg dm^{-3} NAA.

Two experiments were undertaken using differing amounts of the two hormones. In the first, a hormone grid was applied to investigate the effect of differing hormone levels on callus initiation, and in the second, plantlet formation from mother callus was determined. In an attempt to speed up callus proliferation, the effect of different agar concentrations was investigated.

Resultant plantlets were 'hardened off' by potting out in

Table 1 Constituents of culture media used for *Anthurium andraeanum* culture; Difco-Bacto Agar — 8 g dm^{-3}, pH adjusted to 5,8 before autoclaving

	Composition			
Medium	Macro elements	Micro elements	Organic	Additional
Murashige & Skoog (1962)	Full strength	Full strength	Full strength (except IAA, kinetin, myoinositol were omitted)	30 g dm^{-3} sucrose; 5 mg dm^{-3} BA; 0,1 mg dm^{-3} NAA
Modified Murashige & Skoog	Half strength	Full strength (except H_3BO_3 & $MnSO_4$ half strength)	As above	30 g dm^{-3} sucrose; 5 mg dm^{-3} BA; 0,1 mg dm^{-3} NAA
Linsmaier & Skoog (1965)	Full strength	Full strength	Full strength (except IAA, kinetin, myoinositol, optional constituents were omitted).	30 g dm^{-3} sucrose; 5 mg dm^{-3} BA; 0,1 mg dm^{-3} NAA
Miller (1965)	Full strength	Full strength	Full strength	30 g dm^{-3} sucrose; 5 mg dm^{-3} BA; 0,1 mg dm^{-3} NAA
	Half strength	Half strength	Half strength	As above.

a peat compost and growing them in a mist house with low light intensity (40 $\mu E\ m^{-2}\ s^{-1}$), or using a plastic bag over the pot to create a humid environment. The plantlets were kept in this humid environment for two months before being transferred to the greenhouse or shadehouse.

Results

Of the five media used for *Anthurium* culture, the modified Murashige & Skoog (1962) medium proved the best for explant growth. For callus production phloem tissue was necessary in the explants. Table 2 summarizes the response of explants in culture using the modified Murashige & Skoog medium. Callus production from leaf explants was greatest when the major vascular trace was included. Young leaf tissue with no vascular traces did not produce callus. The petiole proved to be the most useful explant for callus production as it always contained vascular tissue. However, callus production from the petioles was much slower (5 – 6 weeks) than callus derived from the leaves (2 – 4 weeks).

Although few root explants survived sterilization, those which did, produced callus originating from the central vascular core after an extended period of time (10 weeks).

Results from a benzyladenine (BA) and naphthylacetic acid (NAA) hormone grid showed that it was not the amount of hormone present in the media which stimulated callus growth, but rather the ratio of cytokinin to auxin. A ratio of cytokinin to auxin of between 50:1 and 10:1 was necessary for callus initiation in anthuriums. Outside this range no callus formation was recorded.

A culture temperature of 20°C resulted in the death of most of the explants within the first week of culture. Those explants which survived were chlorotic. Table 3 lists the responses of the explants to the varied culture environments. Contrary to previous reports (Pierik *et al.* 1975; Pierik 1976), it was noticed that callus production was better in a light environment. Of the explants maintained in continuous dark, only 26% produced callus. However when transferring the dark grown explants to the light/dark cycle, callus was initiated within a week.

Using the modified Murashige & Skoog (1962) medium with 0,7% agar, increased callus proliferation was seen above that recorded for the standard culture medium as well as that for a liquid medium. Shoot proliferation occurred on media with both high cytokinin and auxin concentrations (5 mg dm^{-3} BA; 0,1 mg dm^{-3} NAA) as well as media with low cytokinin and auxin concentrations. Media with lower hormone levels resulted in earlier shoot production, but reduced the number of shoots formed. Root initiation occurred spontaneously 2 – 3 weeks after shoot initiation. A modified medium with associated culture environment was not essential for root production. Root production frequently occurred on callus before shoot production, particularly in media with high cytokinin concentrations.

Once the first 'crop' of plantlets had been removed from the mother callus the remaining callus could be maintained on a medium without hormones. In addition new shoots and

Table 2 A comparison of the efficiency of various plant organs for *Anthurium* tissue culture. One percent NaOCl was used as sterilant. (± indicates the standard error)

Organ used for explants	Explants decontaminated and surviving (%)	Callus formation by explants (%)
Leaf		
Major vein	80 ± 16	54 ± 15
Minor vein	86 ± 9	50 ± 20
Petiole	73 ± 8	72 ± 17
Spathe	0	0
Spadix	0	0
Root	5 ± 5	5 ± 5

Table 3 Response of *Anthurium* explants to variation in the culture environment (± indicates the standard error)

Treatment	Plants decontaminated and surviving (%)	Explants surviving with callus (%)
16-h light: 8-dark 25°C	88 ± 12	66 ± 18
16-h light: 8-dark 20°C	40 ± 20	11 ± 9
Continuous dark 25°C	83 ± 7	26 ± 3
Continuous dark 20°C	33 ± 28	0
Continuous light 25°C	94 ± 14	53 ± 18

roots were produced within 10 days without any further subculturing. The production of plantlets on the mother callus occurred throughout the year for which the experiment was run. It appears that after the initial callus stimulation, hormonal supplements are no longer necessary for plantlet formation.

Figures 1 – 3 illustrate the multiple production of plantlets, and their development at various stages in culture.

Discussion

Contrary to previous reports where very young and soft tissue was reported to be an 'absolute requirement' for callus induction (Pierik *et al.* 1974), it was found that fully expanded leaves and not the 'just unfolded soft leaves' (Pierik *et al.* 1979) had a high regeneration capacity. Vascular tissue and particularly the presence of phloem and associated meristematic tissue was necessary for callus production. Phloem tissue is metabolically active and capable of growth in culture, as well as retaining some of its endogenous growth factors for additional stimulation of explant growth. Callus, derived from root explants of *Anthurium andraeanum*, and subsequent development into plantlets, has not previously been reported in the literature. Although the root explant is difficult to decontaminate, it is a potential source of regenerative tissue.

Use of a culture environment with low light intensity proved to be more stimulatory to callus growth and subsequent plantlet development than continuous darkness. Pierik *et al.* (1974, 1975), and Pierik (1976), reported that for all phases of culture, continuous darkness was advantageous for growth of explants. From the present results it appears that continuous

Figure 1 Three stages in *Anthurium* tissue culture; (a) shoot-producing mother callus; (b) isolated shoot; (c) 'mature' plantlets ready to be potted out.

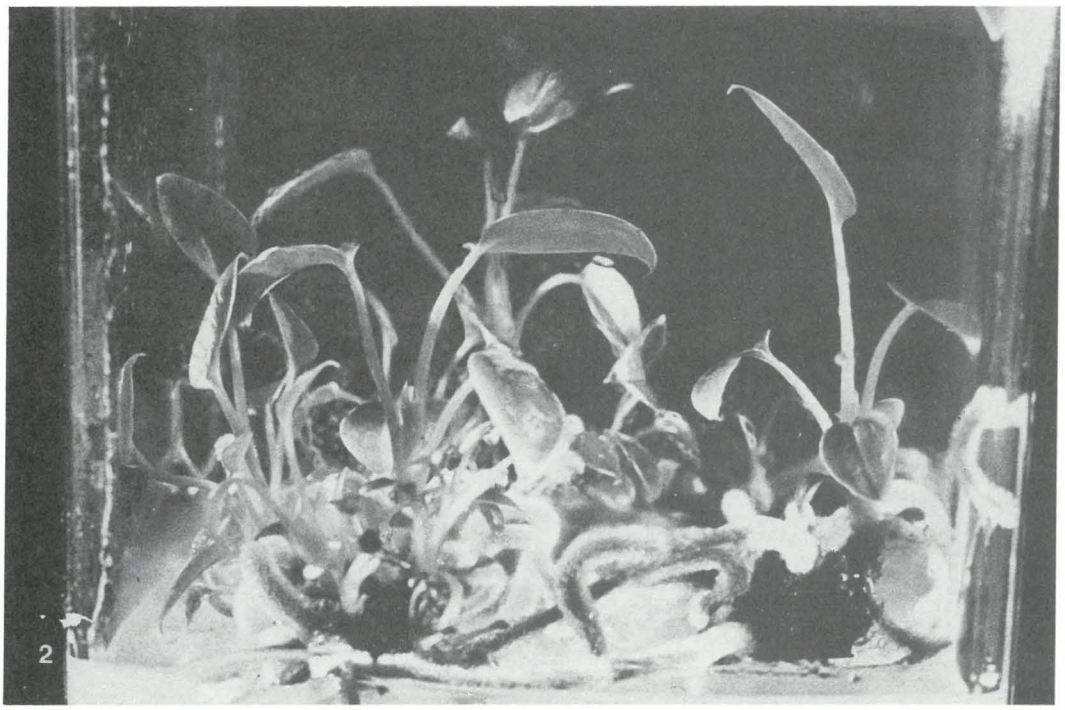

Figure 2 Multiple plantlet production from callus.

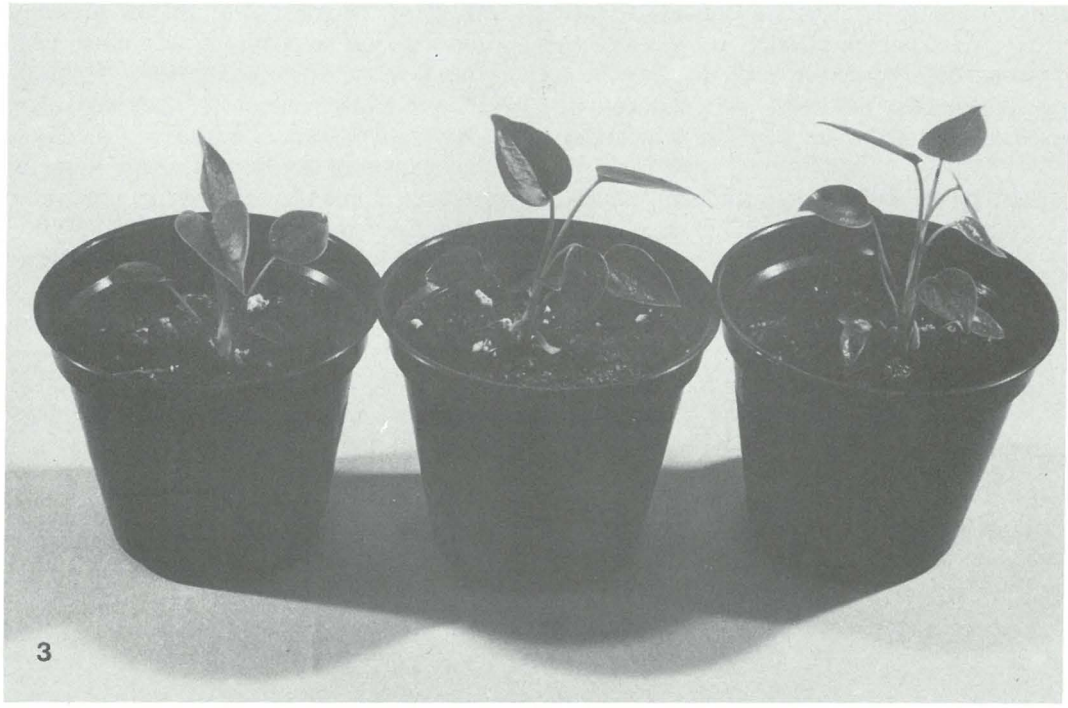

Figure 3 Plantlets produced after the final stage of tissue culture.

darkness has a slight inhibitory effect, whereas a low light intensity (27 $\mu E\ m^{-2}\ s^{-1}$) allows normal metabolic functioning without inhibition of callus induction.

Previous studies (Pierik *et al.* 1974; Leffring & Soede 1979) all used 'soft' media (0,7% agar) for all aspects of *Anthurium* culture. The present study indicates that a 'soft' medium is more stimulatory for callus proliferation than a harder induction medium or the liquid proliferation medium. It appears from these experiments that the firmness of the medium may have a significant effect on the type of growth (induction and sprout formation) of the explant, as well as its performance in culture.

The present results show that the same media can be used for all stages of *Anthurium* tissue culture, from callus induction through to plantlet formation. This appears unique in that the propagation steps set out by Murashige (1974) are not applicable. All *in vitro* propagation stages can be performed without complex environmental or nutritional modifications. Pierik *et al.* (1979) stated that regeneration of leaf explants of *Anthurium adraeanum* is limited both qualitatively and quantitatively by a complex of factors: plant, environmental, nutritional and hormonal. Although the medium used may not be optimal for all stages of culture, the explant requires only an initial input of hormones. Provided with the correct culture environment, the explants appear capable of producing their own growth requirements without further hormonal supplementation.

A single medium which can be used for all phases of *Anthurium* growth in culture will be of importance for commercial growers of this plant as it will eliminate the use of complex culture media, involved sterilization procedures and environmental manipulation. The duration of the cycle from leaf explants through to complete plantlet formation can be reduced from 12 months as proposed by Pierik (1976) to between 7 – 8 months.

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